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In vitro DNA damage prevention, antioxidant and antidiabetic activities of achillea biebersteinii/millefolium extracts and synthesized ZnO nanoparticles

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Abstract

Aim: In this study, DNA damage preventive effects, antioxidant and antidiabetic activities of extracts of Achillea species growing endemic in the Lapta region of Kyrenia district of Cyprus, and zinc oxide-nanoparticles/Achillea (ZnO-NPs/Ach) were investigated.

Material and Methods: We performed green synthesis of ZnO-Nps using the extract obtained from Achillea species (ZnO-NPs/Ach). We performed green synthesis of ZnO-Nps using the extract obtained from Achillea species (ZnO-NPs/Ach). Additionally, we determined the anti-DNA damage, antioxidant, and antidiabetic activities of these nanoparticles.

Results: The highest concentration of 500 µg/mL of ZnO-NPs/Ach had an excellent protective effect. It was determined that the activity of ZnO-NPs/Ach was relatively vigorous in preventing lipid peroxidation. The lipid peroxidation inhibitory activities of ZnO-NPs/A. biebersteinii and ZnO-NPs/A. millefolium were calculated as 90.87% and 89.11%, respectively. Additionally, both demonstrated high antidiabetic effects compared to the positive control acarbose.

Discussion: As zinc oxide nanoparticles obtained through green synthesis have been found to have preventive effects on DNA damage, as well as antioxidant and antidiabetic properties, this study is expected to make a valuable contribution to the medical and technological literature.

Keywords

DNA Damage Prevention, Antioxidant, Antidiabetic, Achillea Biebersteinii, Achillea Millefolium, Green Synthesis, ZnO Nanoparticles

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Introduction

Studies in the health field worldwide have shown that nanoscience has made significant contributions to medicine, particularly in the diagnosis, treatment, and prevention of diseases, as well as drug development. Recently, nanoparticles have been utilized in the diagnosis and treatment of various diseases, including as antimicrobial, antioxidant, antifungal, anticancer, and antidiabetic agents. Green synthesis is the preferred method for obtaining nanoparticles due to its cost-effectiveness, environmental friendliness, ease of preparation, and controllability, as opposed to the use of decaying reaction conditions and hazardous intermediate components.

Zinc oxide nanoparticles (ZnO-Nps) are particularly advantageous compared to other metal oxide nanoparticles due to their electrical and optical properties, biocompatibility, low cost, and low toxicity. Additionally, they have the potential to exhibit antibacterial and cytotoxic effects against cancer cells. ZnO-NPs are frequently used by researchers due to their wide range of applications in medicine and health science.

The genus Achillea is cultivated in temperate zone countries, including Cyprus, Türkiye, Iran, and Pakistan. Extracts from Achillea species, such as A. biebersteinii and A. millefolium, have been traditionally used in folk medicine in those countries for their diuretic, appetizing, gas-digesting, wound healing, astringent (especially in haemorrhoids), urinary antiseptic, and antitussive properties. It is also known to be successfully applied in treating inflammatory conditions and pain, such as menstruation and postpartum discomfort [1,2].

This study aims to perform green synthesis of ZnO-Nps using the extract obtained from Achillea species (ZnO-NPs/Ach). Additionally, the study will investigate the anti-DNA damage, antioxidant, and antidiabetic activities of these nanoparticles. The results of this analysis will contribute to the field of green synthesis studies in nanotechnology.

Material and Methods

Preparation of Achillea species extracts

A. biebersteinii and A. millefolium were collected from Lapta Hill in Kyrenia, Cyprus. The plant samples were identified, and a voucher specimen was recorded and stored in the Herbarium of Girne American University for reference. The Achillea species studied were dried and powdered using an electric plant grinder. All chemicals used in the study, zinc acetate ($Zn(CH_3COO)2.2H_2O$), were purchased from Merck Company (Germany). The powdered A. biebersteinii/millefolium (200 g) was extracted using 2 L of 70% ethanol as a solvent in a 1:10 ratio for 24 hours with regular shaking. The resulting mixture was filtered using a Whatman 1 filter paper. The ethanolic extracts of A. biebersteinii/millefolium plant were stored at +4°C in a refrigerator for further research [3].

Synthesis of ZnO-NPs/Ach

Firstly, 100 mL of A. biebersteinii/millefolium extract and 500 mL of 1 MM zinc acetate solution were left at room temperature for 1 hour. The colour of the mixture changed from yellow to brown, indicating the formation of ZnO-NPs/Ach. The solution was then repeatedly centrifuged (10,000 rpm x 10 minutes) and distilled water was added. The solid particles obtained from centrifugation were filtered and dried in the oven at 50°C for 24 hours. The absorbance of ZnO-NPs/Ach was measured using a UV spectrophotometer (Shimadzu UV 1800, Japan) in the wavelength range of 200-790 nm [4]. The structural characterisation and particle size examination of ZnO-NPs/Ach were done by Transmission Electron Microscopy (TEM) and Scanning Electron Microscopic (SEM) (Hitachi, Japan). In addition, the spectra properties of those were carried out by Fourier Transform Infrared Spectroscopy (FTIR) (Bruker, Germany) in the spectra range of 4000–400 cm^{-1}. X-ray diffraction (XRD) of the ZnO-NPs/Ach was analysed using an XPert PRO diffractometer (Holland) in the 2θ range of 20–80°

The preventive effect of ZnO-NPs/Ach on DNA damage

To investigate the protective effect of ZnO-Nps/Ach against DNA damage, electrophoresis was performed using PBR322 plasmid DNA (4361 base pairs) [5]. A 1% agarose gel was loaded into five wells. The first well contained plasmids and dye, while the seven wells. contained plasmids and loading dye exposed to hydrogen peroxide and UV rays for DNA damage. Plasmids, loading dye, hydrogen peroxide, and ZnO-NPs/Ach solutions were loaded from the third to seventh well in different concentrations (50-100-150-250 and 500 µg/mL). Electrophoresis was then performed to obtain images (Table 1).

Antioxidant activity of ZnO-NPs/Ach

The lipid peroxidation inhibitory activity of ZnO-NPs/Ach was determined using the thiobarbituric acid (TBA) test [6]. To prepare the ZnO-NPs/Ach solution the concentration series (50, 100, 150, 250, and 500 mg/L [w/v]) were dissolved in 97% ethanol. Next, 200 µL of ZnO-NPs/Ach, 200 µL of $FeCl^3$, 200 µL of EDTA, 200 µL of H_2O_2, and 200 µL of ascorbic acid were added and mixed with a vortex. The tubes were then incubated at 37°C for 1.5 h. After incubation, 1.2 mL of 28% TBA was added and the mixture was centrifuged at 3000 rpm for 15 minutes. The remaining pellet was then filtered and 1.2 mL of TBA was added again. The tubes containing the samples were boiled for 10 minutes and then cooled to room temperature. The absorbances of the samples were measured at 532 nm using a spectrophotometer. The lipid peroxidation inhibition was calculated using the formula: inhibition = [(Acont - Asamp) / Acont] × 100 (Eq-1).

Antidiabetic effects of ZnO-NPs/Ach

To investigate the antidiabetic effects of Ach spp, we assessed the enzyme inhibition capacities of the carbohydrate digestive enzymes α-amylase and α-glucosidase [7]. For the α-amylase test, the enzyme solution was prepared by mixing 27.5 mg of alpha-amylase (Sigma Aldrich Chemical Co, USA) into 100 mL of distilled water. 1 mL of the α-amylase enzyme (1 U/mL), 0.1 g/mL ZnO-NPs/Ach and 10 mL of 6.85 mM NaCl (pH 6.9 adjusted using a phosphate buffer) were mixed and centrifuged at 8,500 rpm for 15 minutes. The liquid was separated, and a 1% starch solution of 500 µL in 0.02 M sodium phosphate buffer (pH 6.9) was added to tubes containing five different concentrations (100-500 µg/mL). The reaction was stopped using a colour reagent, 3.5-dinitrosalicylic acid-DNS, and the samples were incubated in a water bath at 100°C for 5 minutes. The tubes were then incubated at 25°C for 10 minutes. After cooling to 20°C, 10 mL of distilled water was added to each sample. Absorbance measurements were taken at a wavelength of 540 nm. The α-glucosidase inhibition capacity was also

determined. Then, we incubated 1 mL of starch substrate (2% maltose or sucrose), 1 U/mL of α-glucosidase enzyme (Sigma Aldrich Chemical Co, USA), and ZnO-NPs/Ach samples (50, 100, 150, 250, and 500 mg/L [w/v]) at 37°C for 5 minutes. The mixture was then placed in a boiling water bath for 2 minutes and cooled to 20C. Absorbance values were measured at 420 20°C nm. We calculated the α-amylase and α-glucosidase inhibitor activity using Eq-1.

Ethical Approval

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article. The ethics committee of Girne American University has stated that there is no need to obtain ethics approval for this reason (Date: 2023-06-06, No: 2023/032).

Results

Synthesis of ZnO-NPs/Ach

The study synthesised ZnO-NPs/Ach from A. biebersteinii/millefolium extracts using green chemistry method. UV spectroscopy, FTIR, XRD, and SEM data were analysed. ZnO-NPs/Ach formation was analysed using a UV spectrophotometer in the 380-410 nm absorbance range, as previous studies have shown [8]. In this study, peak values were determined at 405 nm, supporting the synthesis of ZnO-NPs/Ach. The FTIR results indicate that ZnO-NPs/Ach exhibit tensile vibrations in the bands between 440 and 540 cm^{-1}. It is common for metal nanoparticles, including ZnO oxides, to exhibit vibration frequencies below 1000 cm^{-1}. Additionally, peaks in the 450 to 600 cm^{-1} range were observed for ZnO-Nps/Ach. Previous studies have reported that C-H molecules (e.g. CH_3, CH_2) in chemical compounds produce peaks in the range of 3500-2800 cm^{-1}. Additionally, stress vibrations of the aromatic groups C=O and C=C in A. biebersteinii/millefolium plants are in the range of 1000-1700 cm^{-1} [9] (Figure 1).

XRD analysis was conducted to determine the crystal structure of the ZnO-Nps/Ach synthesized in our study. The peaks in Figure 2 were identified as 34.38, 37.22, 44.79, 49.82, 55.91, 63.04, 65.57, 68.32, and 77.34 for A. biebersteinii and 35.02, 42.21, 50.08, 57.33, 62.56, 69.26, and 76.92 for A. millefolium. The XRD peaks identified in our study were consistent with the results found in previous studies [10], verifying the hexagonal structure for the nanoparticles synthesized in cross-lattice planes (002), (100), (101), (102), (103), (110), (112), (200), and (202).

The synthesis of ZnO-Nps/Ach is confirmed by the zinc peak observed in the EDX spectrum. The carbon, potassium, and magnesium peaks, however, are attributed to bioactive components attached to the surface of ZnO-Nps. The SEM analysis determined that the synthesized ZnO-Nps/Ach are evenly distributed and not clustered in specific regions [10] (Figure 2).

Anti-DNA damage activities of ZnO-NPs/Ach

The study investigated the protective effect of ZnO-NPs/Ach against DNA damage using agarose gel electrophoresis and PBR322 plasmid DNA as target DNA. Different concentrations of zinc nanoparticles (50-100-250-150-500 µg/mL ZnO-NPs/Ach) were compared in wells. The DNA progressed in the first well but was damaged by H2O2 and UV from the second well onwards. The gel electrophoresis results indicate that the addition of 50 mg/L nanoparticles in the third well prevented DNA damage, and as the ZnO-NPs/Ach ratio increased, the DNA moved more significantly. Furthermore, the addition of ZnO-NPs/Ach after the third well prevented DNA damage as the ZnO-NPs/Ach concentration increased. The highest concentration of 500 µg/mL of ZnO-NPs/Ach in the seventh well showed an excellent protective effect. Figure 3 shows the agarose gel electrophoresis.

Antioxidant activity of ZnO-NPs/Ach

We found that the lipid peroxidation prevention activities of ZnO-NPs/Ach increased in direct proportion to the concentration. The highest inhibition value was observed at the nanoparticle level of 500 µg/mL, as determined by the TBA assay. The MDA levels of ZnO-NPs/A. biebersteinii and ZnO-NPs/A. millefolium were found to be 90.87% and 89.11%, respectively (see Figure 6).

Antidiabetic effects of ZnO-NPs/Ach

The antidiabetic effect of ZnO-NPs/Ach was compared to that of acarbose (Sigma Aldrich Chemical Co, USA), which was a

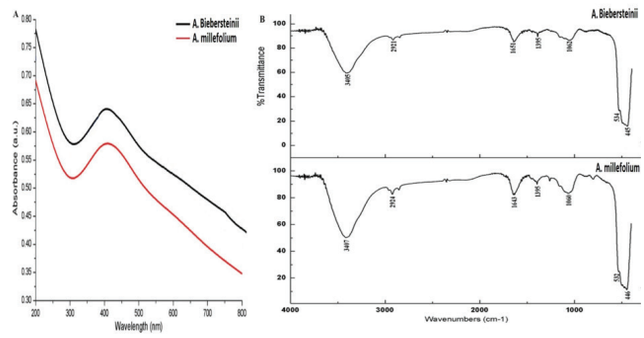

Figure 1. A: UV, and B: FTIR spectra of ZnO-Nps/Ach synthesised by A. biebersteinii/millefolium

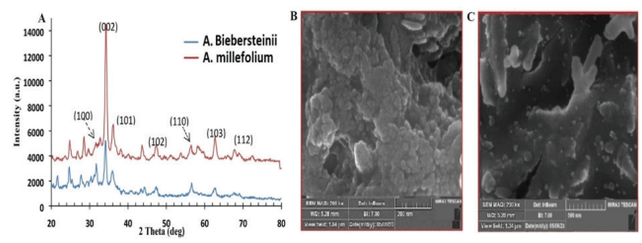

Figure 2. A: XRD spectra for ZnO-Nps/Ach, and SEM images of ZnO-Nps for A. biebersteinii (B) and A. millefolium (C)

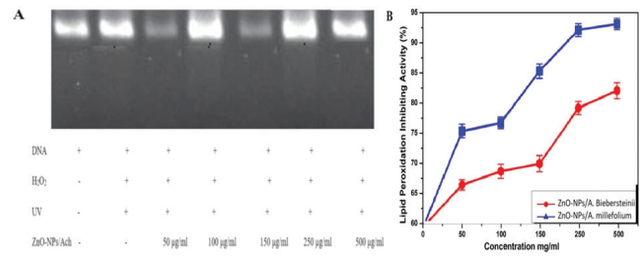

Figure 3. A: Agarose gel electrophoresis for ZnO-NPs/Ach, and B: Lipid peroxidation inhibitory activity of ZnO-NPs/Ach

Table 1. The pit contents were placed in the agarose gel for electrophoresis

	1. Pit	2. Pit	3. Pit	4. Pit	5. Pit	6. Pit	7. Pit
Loading dye	+	+	+	+	+	+	+
ZnO-NPs/Ach	–	–	50 µg/mL	100 µg/mL	150 µg/mL	250 µg/mL	500 µg/mL
DNA	+	+	+	+	+	+	+
H_2O_2	-	+	+	+	+	+	+
UV	–	+	+	+	+	+	+

Table 2. The inhibition activity of α-amylase/α-glucosidase was studied using ZnO-Nps/Ach

Sample	α-amylase inhibition IC50 (µg/mL)	α- glucosidase inhibition IC50 (µg/mL)
Acarbose (standard)	224.82 ± 7.62	160.54 ± 4.37
ZnO-NPs/A. biebersteinii	477.05 ± 3.57[a]	242.28 ± 3.25[a]
ZnO-NPs/A. millefolium	485.48 ± 5.21[a]	251.89 ± 6.32[a]

Mean values were obtained from three parallel measurements ± SEM (n = 3). [a] The significant difference between the standard and experimental groups was determined using one-way ANOVA followed by Tukey's test (p≤ 0.05).

standard. Acarbose inhibited the α-amylase and α-glucosidase at 64% and 88.52%, respectively. ZnO-NPs/A. biebersteinii and ZnO-NPs/A. millefolium had effects of 58.12% and 52.55%, and 94.37% and 92.57%, respectively (Table 2).

Discussion

Reactive oxygen species (ROS) formed in the body can cause rapid oxidation of target molecules, leading to lipid peroxidation in cells, among other biochemical reactions. This process can contribute to various diseases caused by oxidative stress, including neurological diseases, aging, cancer, and heart disease [11,12]. Lipid peroxidation can result in the formation of complex compounds containing reactive carbonyl compounds, such as malondialdehyde (MDA). The MDA measurement is commonly used as a marker of lipid peroxidation in studies related to oxidative stress and redox signalling, particularly in those investigating the antioxidant effects of plants. According to a study [13], medicinal plants can prevent lipid peroxidation through their interaction with ZnO. The study found that the lipid peroxidation prevention activities of ZnO-NPs/Ach increased in direct proportion to the concentration. The highest inhibition value was observed at the nanoparticle level of 500 µg/mL.

Our study aimed to determine the potential antidiabetic effect of Achillea spp., a medicinal plant used in the treatment of various diseases, including diabetes, in Türkiye and Cyprus. These plants exert their antidiabetic effects by controlling hyperglycaemia through the inhibition of enzymes such as α-amylase and α-glucosidase, as well as delaying glucose absorption [14,15]. Acarbose was used as a standard, and it was found to have a strong inhibitory activity against α-amylase and α-glucosidase when compared to ZnO-NPs/Ach.

Cancer is caused by mutations in cells. Preventing diffraction and mutation of the DNA molecule is crucial, particularly in the treatment of pathological processes such as cancer. Recent studies have focused on synthesising nanocomponents and investigating their effects on preventing DNA damage [16]. In this study, we observed that the addition of 50 mg/L nanoparticles prevented DNA damage in the third well. Additionally, we found that the DNA mobility increased as the ZnO-NPs/Ach ratio increased. Furthermore, we observed that the addition of ZnO-NPs after the third well prevented DNA damage as the ZnO-NPs/Ach concentration increased. Finally, we found that the highest concentration of 500 µg/mL of ZnO-NPs/Ach had a perfect protective effect. A study was conducted using electrophoresis of agarose gel to examine the biogenic zinc oxide nanoparticles formed by green synthesis mediated by Thymbra spicata L. The study investigated the effectiveness of DNA cleavage ability and found that the protective effect of ZnO-NPs against DNA damage increased with concentration [17].

Conclusion

Our study investigated the antioxidant effects of ZnO-NPs/A. biebersteinii and ZnO-NPs/A. millefolium, synthesized using the green method. The results showed that the nanoparticles exhibited radical quenching activity and reduced biomolecular agents in plant structure. ZnO-NPs/Ach demonstrated high antioxidant activity, particularly at a concentration of 500 µg/mL. ZnO-NPs/Ach have been found to be potent inhibitors of α-amylase and α-glucosidase, as well as having antidiabetic activity. Additionally, these nanoparticles provide high levels of protection against DNA damage. It has been suggested that ZnO-NPs/A. biebersteinii and ZnO-NPs/A. millefolium could be used as antioxidant or antidiabetic agents to protect cells against DNA damage. Zinc oxide nanoparticles may have potential in the treatment of cancer, diabetes, and other chronic diseases caused by oxidative stress. Our study's results could be a precursor to further scientific research in the health field. More comprehensive studies should investigate the effects of Achillea plant extracts and the nanoparticles synthesized from them.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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